DATA EVALUATION RECORD
SEEDLING EMERGENCE AND SEED GERMINATION EC_{25} TEST
GUIDELINE 123-1a (TIER II)

1. **CHEMICAL:** Dicamba. Shaughnessey No. 029801.

2. **TEST MATERIAL:** Dicamba Technical; CAS No. 1918-00-9; Lot No. 52204112; 89.5% active ingredient.

3. **CITATION:** Hoberg, J.R. 1993. Dicamba Technical - Determination of Effects on Seed Germination, Seedling Emergence and Vegetative Vigor of Ten Plant Species. Laboratory Report No. 93-3-4664. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Sandoz Agro, Inc., Des Plaines, IL. EPA MRID No. 428463-01.

4. **REVIEWED BY:** Michael Davy, Agronomist, EEB

Signature: Michael Davy **Date:** 3/20/95

5. **APPROVED BY:** Daniel Rieder, Section Head, EEB

Signature: Daniel Rieder **Date:** 3-24-95

6. **CONCLUSION:** Seedling Emergence study is scientifically valid but does not meet all of the guidelines for 123-1 (a) for non-target plants. Seed Germination study is scientifically valid and meets all of the guideline requiremnts for a 123-1a Seed Germination study for non-target plants.

Seed Germination

1. Most sensitive monocot: **onion**, Parameter: radicle length
EC_{25}: 0.071 lb ai/A, EC_{50}=0.51 lb ai/A (0.22-1.4)
NOEC=0.032 lb ai/A

2. Most sensitive dicot: **soybean**, Parameter:radicle length.
EC_{25}: 0.036 lb ai/A, EC_{50}=0.26 lb ai/A (0.11-3.9)
NOEC <0.032 lb ai/A

Seedling Emergence

1. Most sensitive monocot: **onion**, Parameter: shoot length
EC_{25}: 0.0424 lb ai/A, EC_{50}=0.1426 lb ai/A (0.06242-0.35873)
slope=0.0424 NOEC <0.032 lb ai/A (Williams test)

2. Most sensitive dicot:
soybean, Parameter:shoot length.
EC_{25}: 0.0027 lb ai/A, EC_{50}=0.0104 lb ai/A (0.00826-0.01334)
slope=1.14778 NOEC <0.0022 lb ai/A (Williams test)

7. **ADEQUACY OF THE STUDY:**
Classification: Supplemental for Seedling Emergence Study
Repairability: None for Seed Emergence Study
Classification: Core for Seed Germination Study

Repairability: N/A for Seed Germnation Study

8. **MAJOR GUIDELINE DEVIATIONS:**
- There were no NOEC's determined for soybean and onion shoot length in seedling emergence study. This would make study suplemental.
- No raw data showing measurements or phytotoxic symptoms per plant in each replicate were submitted
- Three replicates sprayed together do not make a true replicate

9. **MATERIALS AND METHODS:**

A. Test Organisms:

Guideline Criteria	Reported Information
Species: 6 dicots in 4 families including soybean and rootcrop; 4 monocots in 2 families including corn	Soybean, Lettuce, Turnip, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, and Onion.
Number of plants/rep: 10 Number of reps/dose: 3	20 seeds/rep (germination) 10 seeds/rep; (seedling emergence); 3 reps/dose
Source of Seed:	see pp. 79-80 of author's report

Comments: Tier I (corn only) and Tier II for seed germination and seedling emergence. **Seeds planted in sand** for seedling emergence with 0.11% to 0.17% organic matter. Seeds had not been treated with fungicides or insecticides. For the emergence test, the three replicates were sprayed together.

B. Test System:

Guideline Criteria	Reported Information
Solvent used:	none used
Site of test:	growth chamber
Planting Method: species/pot	20 seeds/petrie dish per rep 10 seeds/13 cm diameter pot per pot; one pot or dish per replicate
Method of Application:	250 ml of solution
Watering Method: bottom watering	bottom-watering with nutrients

Guideline Criteria	Reported Information
No. Days After Application: 14 days/emergence; 5 days/germination	14 days for seedling emergence 5 days for seed germination

Comment: Control and treated replicates were segregated by control on lowest shelf then lowest concentration on next shelf and so on with the highest concentration on the top shelf. Air circulation of growth chamber was directed from lower concentration to highest concentration. An earlier testing of dicamba found that volatilization of dicamba affected the other replicates. This arrangement was designed to reduce the impact of such volatilization.

C. **Test Design:**

Guideline Criteria	Reported Information
Dose range No.: 2X or 3X	2X
Controls:negative and solvent	negative only
Parameters Observed/Measured	Seedling Emergence: height, emergence %. Seed Germination: germination %, radicle length.
Maximum Labeled Rate:	2.1 lb ai/A

Comment: Supplemental test for turnip was made to determine NOEC. Sand was treated with test solution and nutrient prior to planting.

10. REPORTED RESULTS:

Guideline Criteria	Reported Information
NOEC Observed?	Yes
Phytotoxic Observations:	morphological descriptions
Measured Initial Chemical Concentrations? Optional	Yes
Raw data included? (Y/N)	No

Author's end point values are in lb ai/A. The parameter bearing the most sensitive EC_{25} values are listed for each plant species. Values in bold denotes the most sensitive monocot and dicot.

Species	Seed Germination[1] EC_{25}/NOEC	Seedling Emergence[2] EC_{25}/NOEC
Cabbage	0.28/0.13	1.5/0.53
Corn	0.78/0.49	0.48/0.25
Cucumber	0.053/0.062	0.42/0.25
Lettuce	0.12/<0.032	0.074/0.13
Oat	0.36/0.25	0.57/0.25
Onion	**0.071/0.032**	**0.0044/<0.032**
Ryegrass	0.19/0.064	0.53/0.25
soybean	**0.036/<0.032**	**0.0027/<0.0022**
tomato	0.031/<0.035	0.054/0.032
turnip	0.20/0.064	0.044/0.016

[1] Values based on the most sensitive parameter in seed germination which is radicle length
[2] Values based on the most sensitive parameter in seedling emergence which is shoot height

Day 14: No. of dead plants out of number of plants emerged

conc.	0.032	0.068	0.14	0.25	0.53	1.0	2.1
cabbage	na	na	0	1/29	5/27	6/19	6/16
corn	na	na	0	0	0	2/29	1/29
cucumber	0	0	0	0	0	1/25	na
lettuce	10/36	14/33	16/36	14/24	na	na	na
oat	na	na	0	0	0	0	0
onion	0	0	0	0	0	na	na
ryegrass	na	na	na	0	0	0	0
soybean	0	na	na	na	na	na	na
tomato	0	0	0	0	na	na	na
turnip	0	0	0	0	0	na	na

Statistical Results Statistical Method: linear regression response and Williams or Kolmogorov-Smirnov tests.

11. **STUDY AUTHORS'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:**
Quality assurance measures were taken.

12. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. Test Procedure:

The study met all of the guideline criteria under the SEP and Subdivision J except for the following :

- There were no NOEC's determined for soybean and onion shoot length

- Plants were planted in sand

- No raw data showing measurements or phytotoxic symptoms per plant in each replicate were submitted

- In the Seedling Emergence test for turnip, seeds for one replicate at 0.016 lb ai/A dose were not planted

- Three replicates sprayed together do not make a true replicate

B. Statistical Analysis: Linear regression analysis and Williams test from EPA's toxanal computer program.

Seedling Emergence

1. Most sensitive monocot: **onion**, Parameter: shoot length
EC_{25}: 0.0424 lb ai/A, EC_{50}=0.1426 lb ai/A (0.06242-0.35873)
slope=0.0424 NOEC <0.032 lb ai/A (Williams test)

2. Most sensitive dicot:
soybean, Parameter:shoot length.
EC_{25}: 0.0027 lb ai/A, EC_{50}=0.0104 lb ai/A (0.00826-0.01334)
slope=1.14778 NOEC= <0.0022 lb ai/A (Williams test)

Seed Germination

1. Most sensitive monocot: **onion**, Parameter: radicle length
EC_{25}: 0.071 lb ai/A, EC_{50}=0.51 lb ai/A (0.22-1.4)
NOEC=0.032 lb ai/A

2. Most sensitive dicot: **soybean**, Parameter:radicle length.
EC_{25}: 0.036 lb ai/A, EC_{50}=0.26 lb ai/A (0.11-3.9)
NOEC= <0.032 lb ai/A

C.Discussion/Results: The plants were planted in sand rather than soil. Over a period of 14 days, plants can show nutrient deficiency symptoms or lack of dicamba concentrations in the sand from leaching out through the

sand. However, it appears that corrective measures were taken by bottom-watering the plants so the dicamba will not move downward in the sand and the nutrients will be available for the plants.

No raw data on individual plants were submitted in which the reviewer could run statistical packages to ascertain the variance between reps and plants. Only summarized and mean data were submitted to determine an EC value.

No NOECs were determined for soybean and onion shoot length because the dosage of dicamba did not go low enough.

The replicates were sprayed together and then randomly placed on different areas of the growth chamber. The reviewer believes that these replicates are not true replicates when treated together.

With all of the deviations combined, especially concerning the lack of NOECs for soybean and onion shoot length (most sensitive parameter), the reviewer feels that this study is scientifically valid but does not fulfill all of the guidelines for 123-1 (a) seedling emergence for non-target plants and is therefore classified as supplemental.

DAVY DICAMBA EMERGENCE SHOOT LENGTH SOYBEAN

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.032	100	68	68	0
.016	100	59	59	0
8.000001E-03		100	47	47
0				
.0063	100	45	45	0
.0022	100	18	18	0

THE BINOMIAL TEST SHOWS THAT 8.000001E-03 AND .016 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.507768E-03

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	6.602596E-02	9.737118E-03	
.007809	1.247786E-02		

$EC_{25} = 0.00269$

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	6.965491E-02	1	.4560812

SLOPE = 1.147776

95 PERCENT CONFIDENCE LIMITS = .8448526 AND 1.4507

LC50 = .0104018

95 PERCENT CONFIDENCE LIMITS = 8.261731E-03 AND 1.333939E-02

LC10 = 8.139934E-04

95 PERCENT CONFIDENCE LIMITS = 3.279346E-04 AND 1.405268E-03

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DAVY DICAMBA EMERGENCE SHOOT LENGTH ONION

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.53	100	83	83	0
.25	100	57	57	0
.14	100	49	49	0
6.800001E-02	100	25	25	0
.032	100	28	28	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1504934

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	8.548934E-02	.16148	.1278518	.2109695

$EC_{25} = 0.04247$

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.4638643	3.562859	1.353467E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.281827
95 PERCENT CONFIDENCE LIMITS = .4088055 AND 2.154848

LC50 = .1425912
95 PERCENT CONFIDENCE LIMITS = 6.242021E-02 AND .3587291

LC10 = 1.456529E-02
95 PERCENT CONFIDENCE LIMITS = 1.102029E-04 AND 4.091843E-02

emergence shoot length onion
File: eme.ono Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	11.200	11.900	11.467
2	0.032	3	7.800	8.900	8.333
3	0.068	3	8.200	8.900	8.567
4	0.14	3	4.900	6.900	5.900
5	0.25	3	3.700	5.900	4.833
6	0.53	3	1.600	2.300	1.867

emergence shoot length onion
File: eme.ono Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.143	0.379	0.219
2	0.032	0.303	0.551	0.318
3	0.068	0.123	0.351	0.203
4	0.14	1.000	1.000	0.577
5	0.25	1.213	1.102	0.636
6	0.53	0.143	0.379	0.219

emergence shoot length onion
File: eme.ono Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	11.467	11.467	11.467
2	0.032	3	8.333	8.333	8.450
3	0.068	3	8.567	8.567	8.450
4	0.14	3	5.900	5.900	5.900
5	0.25	3	4.833	4.833	4.833
6	0.53	3	1.867	1.867	1.867

emergence shoot length onion
File: eme.ono Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	11.467				
0.032	8.450	5.290	*	1.78	k= 1, v=12
0.068	8.450	5.290	*	1.87	k= 2, v=12
0.14	5.900	9.762	*	1.90	k= 3, v=12
0.25	4.833	11.632	*	1.92	k= 4, v=12
0.53	1.867	16.835	*	1.93	k= 5, v=12

s = 0.698

Note: df used for table values are approximate when v > 20.

emergence shoot length soybean
File: eme.soy Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	43.100	47.600	45.900
2	0.0022	3	36.700	38.800	37.600
3	0.0063	3	22.900	27.500	25.433
4	0.0080	3	21.900	26.600	24.500
5	0.016	3	17.500	20.500	19.033
6	0.032	3	14.300	14.700	14.500

emergence shoot length soybean
File: eme.soy Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	5.970	2.443	1.411
2	0.0022	1.170	1.082	0.624
3	0.0063	5.453	2.335	1.348
4	0.0080	5.710	2.390	1.380
5	0.016	2.253	1.501	0.867
6	0.032	0.040	0.200	0.115

emergence shoot length soybean
File: eme.soy Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	45.900	45.900	45.900
2	0.0022	3	37.600	37.600	37.600
3	0.0063	3	25.433	25.433	25.433
4	0.0080	3	24.500	24.500	24.500
5	0.016	3	19.033	19.033	19.033
6	0.032	3	14.500	14.500	14.500

emergence shoot length soybean
File: eme.soy Transform: NO TRANSFORMATION

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	45.900				
0.0022	37.600	5.487	*	1.78	k= 1, v=12
0.0063	25.433	13.529	*	1.87	k= 2, v=12
0.0080	24.500	14.146	*	1.90	k= 3, v=12
0.016	19.033	17.760	*	1.92	k= 4, v=12
0.032	14.500	20.756	*	1.93	k= 5, v=12

s = 1.853

Note: df used for table values are approximate when v > 20.

SLOPE = 1.147776 LC50 = .0104018 LC25 =
2.690343E-03
SLOPE = 1.281827 LC50 = .1425912 LC25 =
4.246615E-02